ISPH-0595 PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Appln. No. : 09/923,515 Confirmation No.: 1714

Applicant : Crooke et al.

Filed: : August 7, 2001

TC/A.U. : 1635

Examiner : T. Gibbs

Customer No. : 36441

Title : MODULATION OF APOLIPOPROTEIN(A) EXPRESSION

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37 CFR § 1.132 DECLARATION

Sir:

I, Mark J. Graham, residing at 2305 S. Ola Vista, San Clemente, California, 92672, a citizen of the United States of America, do hereby declare and state that:

1. I am one of the named joint inventors of the subject matter claimed in the above-captioned patent application. I earned a Bachelor of Science degree from Rockhurst University in 1982. I have been employed at ISIS Pharmaceuticals since 1991 and am currently an Associate Director of the Cardiovascular Antisense Drug Discovery Group.

2. This Declaration is submitted in the above-identified application in response to the Examiner's rejection under 35 USC 103(a) over McLean et al. (Nature, 330:132 (November 12, 1987) in view of Prosnyak et al. (Genomics, 3:490-494 (1994)) and Deverre et al. (Nucl. Acids. Res., 25:3584 (1997)) in the Office Action mailed April 6, 2005. I have personally reviewed the documents described in this paragraph.

3. In this rejection, the Examiner asserted that McLean discloses a 30-base oligonucleotide probe that spans the breakpoint of apo(a) and plasminogen (Figure 1b, dotted underline) which is reverse complementary to nucleobases 80-109 of SEQ ID NO: 3 of the instant invention.

4. Applicants respectfully rebut the Examiner's assertion in point 3. As credentialed above, I believe that I am a typical person of skill in the art with knowledge of cDNA cloning and probe hybridization. I have reviewed McLean in detail. In my opinion, McLean does not teach that the 30-base primer in Figure 1 and identified in point 3 is reverse complementary to any portion of SEQ ID NO: 3, including nucleobases 80-109 of SEQ ID NO: 3, of Applicants' invention.

5. Evidence in the form of known laboratory procedures of cDNA cloning and probe hybridization further support this assertion that McLean does not teach a 30-base oligonucleotide that is reverse complementary to nucleobases 80-109 of SEQ ID NO: 3.

6. In my opinion, McLean does not provide any teaching of any reverse complementary oligonucleotide probes. In fact, McLean only discusses a 30 nucleobases oligonucleotide that is 100% identical to nucleobases 80-109 of SEQ ID NO: 3, ***but is not reverse complementary*** to the same portion of SEQ ID NO: 3. Nor does McLean even suggest any other oligonucleotide probe.

7. Conventional knowledge in the art teaches that when cDNA libraries are probed, the exact nucleic acid sequence of the desired target is typically used. This knowledge in the art is evidenced by the description of the procedure of cDNA cloning and probe hybridization contained in Maniatis, "Molecular Cloning, A Laboratory Manual", Cold Spring Harbor Press, 1989, which is a manual utilized by a large number of molecular biology laboratories.

8. In this document utilized by those skilled in the art, Maniatis specifically discusses that cDNA libraries are constructed using the RNA from the tissue of interest, e.g., liver tissue in the case of McLean. The RNA provides the template for a reverse transcriptase reaction, which produces the complementary DNA strand (cDNA). This cDNA then becomes the template for a DNA polymerase reaction, thereby resulting in a double-stranded cDNA. The double-stranded cDNA is then probed in the library screening process.

Because both DNA strands are present in the library, the probe is not necessarily the reverse and

complement of the target sequence. In fact, synthetic oligonucleotide probes usually correspond to part of the sequence of a segment of DNA and in general "such oligonucleotides match with their target sequence perfectly"[1].

9. I hereby declare that all statements made herein are of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date: 6/22/05 By: Mark J. Graham

[1] Sambrook, J., Fritsch, EF, and Maniatis, T., in Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory Press, NY, Vol. 2, (1989), Chapter 11, page 4, line 3.

SINGLE OLIGONUCLEOTIDES OF DEFINED SEQUENCE

Probes consisting of a single oligonucleotide of defined sequence usually correspond to part of the sequence of a previously cloned segment of DNA. In general, such oligonucleotides match with their target sequence perfectly, or nearly perfectly, and they are sufficiently long (19–40 nucleotides) to allow the use of hybridization conditions that can guarantee discrimination between the target sequence and other closely related sequences. Single oligonucleotides of defined sequence are used:

- To screen cDNA or genomic DNA libraries, or subclones derived from them, for additional clones containing segments of DNA that have been isolated previously and sequenced
- In Southern, northern, or dot-blot hybridization to identify or detect the sequences of specific genes
- In Southern or dot-blot hybridization of genomic DNA to detect specific mutations in genes of known sequence
- To detect specific mutations generated by site-directed mutagenesis of cloned genes
- To map the 5′ termini of mRNA molecules by primer extension

Oligonucleotide probes used for these purposes are usually labeled by phosphorylation of 5′ termini with [γ-^{32}P]ATP (see pages 11.31–11.32) or, more rarely, by synthesis of a radioactive complementary strand with the Klenow fragment of *E. coli* DNA polymerase I (see pages 11.40–11.44). Because the base composition and size of each oligonucleotide probe are known precisely, conditions can be defined that allow hybridization of the oligonucleotide to its desired target and little or no hybridization to closely related sequences that are not perfectly matched (see below). Obviously, such probes are limited to applications where the sequence of the target DNA or RNA is already known (see, e.g., Montgomery et al. 1978); they are of no use when screening libraries to isolate clones of unknown sequence.